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“Knowledge is such a treasure which cannot be stolen”

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IS : 3509 - 1966

Indian Standard

METHODS OF SAMPLING AND
TEST FOR CREAM

(Second Reprint APRIL 1983)

UDC 637.148 : 543.05



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INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

Indian Standard

METHODS OF SAMPLING AND TEST FOR CREAM

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Indian Standard

METHODS OF SAMPLING AND TEST FOR CREAM

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 3 May 1966, after the draft finalized by the Dairy Industry Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Cream is one of the most important dairy products. It is used as such, or as a raw material for the manufacture of table butter, or for preparing ghee. This standard has been formulated with a view to providing uniform methods of analysis and facilitating the interpretation of results. The methods are also applicable to *MALAI*.

0.3 In the formulation of this standard, considerable assistance has been derived from the following publications:

NEN 2771-1958 Determination of the fat content of cream with the Gerber butyrometer by the 'dilution method'. Nederlands Normalisatie-instituut.

B.S. 696 (Parts 1 & 2) : 1955 Gerber method for the determination of fat in milk and milk products. British Standards Institution.

B.S. 809 : 1963 Methods for sampling milk and milk products. British Standards Institution.

B.S. 1741 : 1963 Methods for the chemical analysis of liquid milk and cream. British Standards Institution.

Methods of analysis of the association of official agricultural chemists. 1960. Ed 9. Association of Official Agricultural Chemists, Washington.

0.3.1 Full use has been made of the valuable information received from the National Dairy Research Institute, Karnal.

0.4 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

*Rules for rounding off numerical values (revised).

1. SCOPE

1.1 This standard prescribes the methods for sampling and analysis of cream and *MALAI*.

NOTE — *MALAI* is that portion of milk which is rich in milk-fat and which rises to the surface of the heated milk on standing.

2. QUALITY OF REAGENTS

2.1 Unless specified otherwise, pure chemicals and distilled water (see IS : 1070-1960*) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

3. SAMPLING

3.1 Sampling shall be carried out by an experienced person as it is essential that sample should be representative of the bulk which may comprise of a large number of packages. Sampling, therefore, requires most careful attention to details if the subsequent analysis is to be of value. As a guide to the selection of sample, useful information may be found in documents and certificates which normally accompany the consignment and usually include classification markings. However, it is recommended that the method given in these documents and certificates should be adhered to wherever practicable.

3.2 Selection of Units for Sampling

3.2.1 *Lot* — All the units in a single consignment belonging to the same batch of manufacture shall be grouped together to constitute a lot. If a consignment consists of different batches of manufacture, the batches shall be marked separately and the group of units in each batch shall constitute separate lots.

3.2.2 *Selection of Units from the Lot* — These units shall be selected at random from the lot. To ensure the randomness of selection, a random number table as agreed to between the purchaser and the supplier shall be used. In case such a table is not available, the following procedure shall be adopted:

Starting from any unit, count them as 1, 2, 3, up to r and so on, in one order, where r is equal to the integral part of N/n , N being the number of units in the lot and n the number of units to be selected. Every r th unit thus counted shall be withdrawn to give required number of units in the sample.

*Specification for water, distilled quality (*revised*).

3.2.3 Bulk Units

3.2.3.1 Units known to be uniform — If cream is supplied in bulk units like churns, and is known to be of uniform quality, the number of units to be selected for sampling shall be as follows:

<i>Number of Units in the Lot</i>	<i>Number of Units to be Selected</i>
<i>N</i>	<i>n</i>
1	1
2 to 9	2
10 „ 49	3
50 „ 99	4
100 „ 199	5
200 „ 999	5 for the first 200 plus one for each additional 200 units, or fraction thereof
1 000 and over	9 for the first 1 000 plus one for each additional 1 000 units, or fraction thereof

3.2.3.2 Units with wide variation — If there is a possibility of wide variations between different units, for example, when a consignment is from individual producers, every unit shall be sampled.

3.2.4 Small Units

3.2.4.1 Units with wide variation — While sampling small units, like bottles and small-tins, incidence of sampling may be varied according to the circumstances. Sampling may also vary according to the information, if any, of the division of the consignment into manufacturing batches. The testing laboratory may be consulted regarding the number and the method of selection of units.

3.2.4.2 Units expected to be uniform — For consignments, or parts of consignments expected to be of uniform quality, the following minimum numbers of units shall be selected at random from separate crates, cases or packages:

<i>Number of Units in the Lot</i>	<i>Number of Units to be Selected</i>
<i>N</i>	<i>n</i>
1 to 100	1
101 „ 1 000	2
1 001 „ 10 000	4
over 10 000	4 for the first 10 000 plus one for each additional 2 500 units, or fraction thereof

3.2.5 The samples shall consist of the unopened retail unit (s) selected.

3.3 Sample Containers

3.3.1 Wide mouth jars and bottles of 50 and 100 ml capacities and following approximate dimensions in millimetres may be used:

<i>Nominal Capacity</i>	<i>Height</i>	<i>Width</i>	<i>Width of the Mouth</i>
50	60	50	30
100	70	60	45

3.3.2 The jar and bottle shall be closed by means of a screw cap lined with butter paper or a glass stopper. For chemical analysis, bottles may be closed with rubber stoppers lined with butter paper if organoleptic tests are not to be made.

3.3.3 Jars, bottles, caps and stoppers shall be suitable for sterilization.

3.3.4 Sample containers shall be perfectly clean and dry, and shall not impart any foreign odour or flavour.

3.3.5 For bacteriological examination, only glass-stoppered bottles shall be used.

3.4 Sampling Appliances

3.4.1 Material — Plungers, dippers and tubes used for sampling cream shall be of stainless steel, or other suitable material of adequate strength and robust construction to prevent distortion in use. They shall, however, be sufficiently light in weight for the operator to be able to move them rapidly through the cream. If solder is used in the manufacture of the sampling appliances, it shall withstand sterilizing temperature of 180°C. All surfaces shall be smooth and free from crevices, and all corners shall be rounded.

3.4.2 Plungers — The plunger shall be of sufficient area to produce adequate disturbance in the unit. A form of plunger recommended as being suitable for mixing cream is shown in Fig. 1. It consists of a disc of 150-mm diameter, perforated with six holes each of 12.5-mm diameter, and a pitch circle of 100-mm diameter, the disc being fixed centrally to a metal rod the other end of which forms a loop handle. The length of the rod, including the handle, should be approximately one metre.

3.4.3 Dippers — Sampling dippers shall be fitted with a solid handle at least 150-mm long and it shall have a capacity of not less than 80 ml. A form of sampling dipper recommended as being suitable is shown in Fig. 2.

3.4.4 Sampling Tube — The sampling tube shall consist of a straight seamless metal tube about 600 mm long of 6 mm inside diameter and about 1.6 mm thickness.

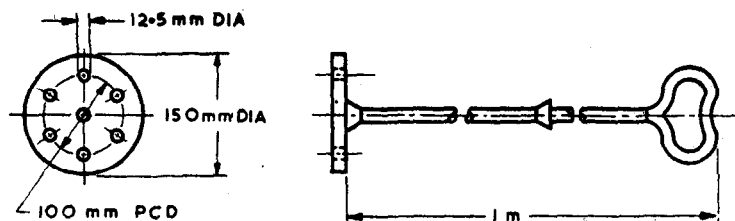


FIG. 1 PLUNGER

3.4.5 All sampling equipment shall be perfectly clean and dry and shall not impart any foreign flavour or odour.

3.5 Mixing of Cream in Containers

3.5.1 When cream is thin and in small containers, it shall be mixed either by six transfers, or by plunging not less than ten times. The position of the plunger shall be moved from place to place to ensure that the whole of the cream at the bottom of the vessel has been thoroughly agitated and mixed with the upper layer. To avoid whipping and churning, the disc of the plunger shall not be brought above the surface of the cream.

3.5.2 When cream is thick or in bulk containers, it shall be mixed by plunging described in 3.5.1.

3.5.3 When the cream is sour, the material shall be warmed so as to attain a temperature between 30° and 40°C and, while cooling it to room temperature, the container shaken gently or the contents stirred. Keep the contents covered as much as possible.

3.5.4 In all cases the sample shall be taken immediately after mixing.

3.6 Preparation of Composite Samples

3.6.1 Taking equal amount of cream from each of the containers selected (3.2.3 or 3.2.4), collect about 300 g of the material which shall be mixed and divided into three parts. One of these samples shall be for the purchaser, one for the vendor and the third for the referee.

3.6.2 The normal size of the final sample (3.6.1) shall be 100 g, but may be less if the examining laboratory so instructs.

3.7 Sampling for Bacteriological Analysis

3.7.1 All sampling equipment, including plungers, dippers, sampling tubes, bottles and stoppers, shall be sterile and the samples shall be collected

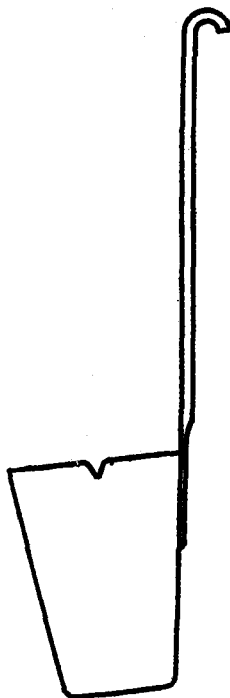


FIG. 2 SAMPLING DIPPER

under aseptic conditions. The equipment shall be sterilized by one of the following methods:

- a) Heating in a hot air for not less than 2 hours at 160°C ; or
- b) Autoclaving for not less than 15 minutes at 120°C .

NOTE 1 — For field conditions, equipment may be sanitized by immersion for at least 5 minutes in boiling water.

NOTE 2 — Rubber stoppers shall be sterilized in an autoclave as in (b). Treatment by immersion in boiling water for 10 minutes would be satisfactory if they are used immediately.

3.7.2 The cream in the containers shall be mixed well as described in 3.5. Immediately after mixing, a sample of about 300 g shall be drawn with a sterilized or sanitized plunger. Transfer directly to a sterilized or sanitized wide-mouthed sample container. Use spoon to assist in removing the cream from the plunger so as not to allow the product to come in contact with the exposed lip of the sample container. The sample shall be divided into three equal parts and placed in three sterilized

or sanitized wide-mouthed sample containers. One sample shall be for the purchaser, another for the supplier and third for the referee. Replace the closure of the sample containers and refrigerate the samples.

3.8 Preservation of Samples

3.8.1 When required for chemical analysis, a suitable preservative may be added if instructed by the examining laboratory. The nature and quantity of such preservative shall be indicated on the label of the container. The preservative shall not interfere with the subsequent analysis. If no preservative is added and a delay in delivery to the examining laboratory is expected, the sample shall be cooled and held at a temperature of 0° to 5°C.

3.8.2 No preservative shall be added to any sample required for bacteriological or organoleptic examination. The sample shall be cooled and maintained strictly within the range of 0° to 5°C.

3.8.3 All samples shall be protected from light and heat, and kept in a cool place.

3.9 Transport and Storage of Sample

3.9.1 Samples shall be sent as quickly as possible to the examining laboratory and shall be protected from light and contaminating odours. The samples shall be cooled in ice and placed in an insulated container capable of maintaining the temperature between 0° and 5°C. Exposure of samples to temperature below freezing point shall be avoided.

3.9.2 Bacteriological examination shall be undertaken within 24 hours of the time of sampling.

3.10 Preparation of Sample for Chemical and Bacteriological Analysis

3.10.1 The preparation of the sample depends upon its physical condition. If, at room temperature, the cream is thin to pour easily, mix by repeated inversion of the container; if it is too thick, stir gently, taking care that the top and bottom layers get well mixed.

3.10.2 It may not be possible to mix by gentle stirring, if the cream is very thick and the fat is partially separated, or if on stirring the cream becomes thick or the fat separates. Under these circumstances, warm the sample to a temperature between 30° and 40°C and, while cooling it to room temperature, shake the container gently or stir the contents at

intervals. Keep the container covered as much as possible to avoid loss of moisture by evaporation.

NOTE — If the sample shows any abnormality, it should be recorded. If satisfactory mixing cannot be achieved, the sample should not be tested.

3.10.3 In the preparation of the sample for bacteriological analysis, suitable aseptic precautions shall be taken to prevent contamination of the sample.

4. DETERMINATION OF FAT

4.1 Gerber Method — For routine work, Gerber method as prescribed in IS : 1224-1958* shall be used.

4.2 Röse-Gottlieb Method

4.2.0 Röse-Gottlieb method shall be used in case of disputes.

4.2.1 Apparatus

4.2.1.1 *Fat extraction apparatus* — as described in IS : 2311-1963†.

4.2.1.2 *Electric oven* — well ventilated and maintained at $100^{\circ} \pm 1^{\circ}\text{C}$.

4.2.2 Reagents

4.2.2.1 *Concentrated ammonia solution* — approximately 35 percent *w/w* (sp gr 0.88). *Concentrated hydrochloric acid* of density 1.122 g/ml at 20°C should be used in place of concentrated ammonia solution if the alternative procedure is used for extraction of fat (see 4.2.3.1).

4.2.2.2 *Ethyl alcohol* — 95 to 96 percent *v/v*, or denatured spirit.

4.2.2.3 *Diethyl ether* — sp gr 0.720, free from peroxide. It may be maintained free from peroxide by adding wet zinc foil (approximately 80 cm² per litre, cut in strips long enough to reach at least half way up the container) that has been completely immersed in dilute acidified copper sulphate solution for one minute and subsequently washed with water.

4.2.2.4 *Light petroleum* — boiling range 40° to 60°C , recently distilled.

4.2.2.5 *Mixed solvent* — prepared by mixing equal volumes of diethyl ether and light petroleum.

4.2.2.6 *Sodium chloride solution* — 0.5 percent (*w/v*).

*Determination of fat in whole milk, evaporated (unsweetened) milk, separated milk, skim milk, buttermilk and cream by the Gerber method.

†Specification for fat extraction apparatus for milk and milk products.

4.2.3 Procedure

4.2.3.1 Extraction using the fat extraction tube (see Fig. 3)

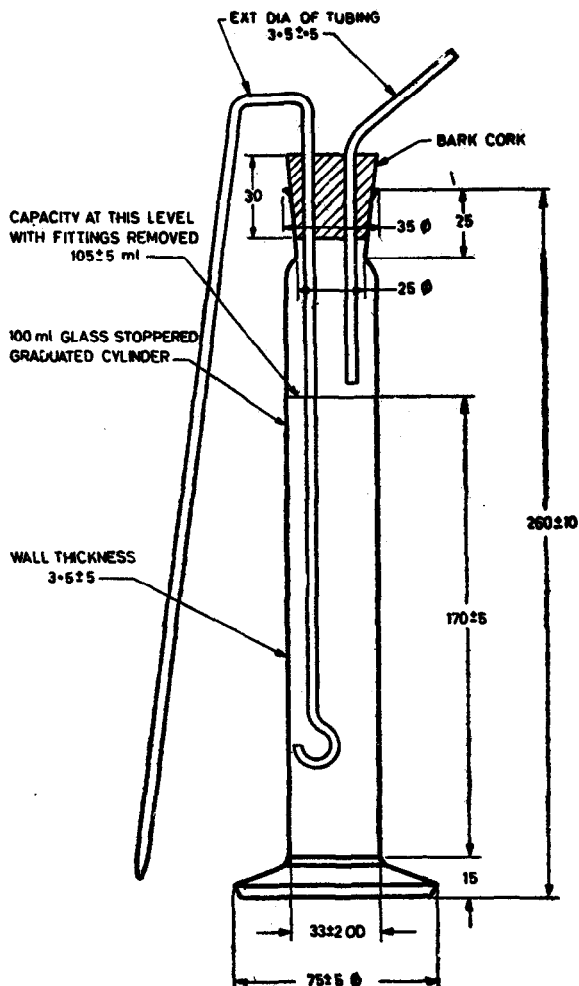
- a) Weigh accurately 1 to 2 g (depending on the fat percentage) of cream into 50-ml beaker. Add 9 ml sodium chloride solution slowly to disperse and transfer to the fat extraction apparatus. Add 1 ml of concentrated ammonia solution and mix well.
- b) Alternately, weigh accurately 1 to 2 g (depending on the percentage) of cream into 50-ml beaker. Add 10 ml of concentrated hydrochloric acid and heat on a water-bath till casein has dissolved. Cool.

With 10 ml alcohol, transfer the contents (a or b) completely to the fat extraction tube and mix well. Add 25 ml diethyl ether through the beaker used for weighing cream. Close the tube with the cork (or stopper), wetted before insertion, and shake vigorously for one minute. The cork (or stopper) shall be wetted with water before each insertion and washed with solvent during each removal. Also, to avoid spurting of the solvent, a slightly reduced pressure should be induced in the tube by cooling before each removal. Rubber stoppers shall not be used. Remove the cork and, with 25 ml of light petroleum, wash the cork and the neck of the tube so that the washings run into the tube. Replace the cork, again wetted with water, and shake vigorously for 30 seconds. Complete extraction of fat is dependent on satisfactory mixing at each stage.

Allow the tube to stand until the ether layer is clear and completely separated from the aqueous layer; this does not take less than 30 minutes. Remove the cork and insert the siphon (or wash-bottle), always keeping the inlet 2 to 3 mm above the interface between the ether and aqueous layers, and transfer the ether layer to a suitable flask containing two small glass beads. Add 5 ml mixed solvent to the extraction tube, using it to wash the siphon (or wash-bottle) fitting, which is not removed but only raised sufficiently for washings. Lower the fittings and transfer the solvent without shaking the flask. Repeat this operation with a further 5 ml mixed solvent. Wash the tip of the siphon fitting into the flask and the neck of the flask with mixed solvent.

Remove the siphon fitting and repeat the extraction of the milk residue, using 15 ml diethyl ether and 15 ml light petroleum, and repeat the subsequent operations as before. Use diethyl ether to wash the inner limb of the siphon (or wash-bottle) fitting during its removal from the tube. Finally, repeat the extraction once more with 15 ml each of diethyl ether and petroleum.

4.2.3.2 Extraction using Mojonnier fat extraction apparatus (see Fig. 4) — Weigh accurately 1 to 2 g (depending on the fat percentage) of the cream into a 50-ml beaker. Add 9 ml of sodium chloride solution. Swirl gently to disperse. With 10 ml ethyl alcohol, transfer the contents to the Mojonnier fat-extraction apparatus. Mix well. Complete extraction of the fat is dependent on satisfactory mixing at each stage.



All dimensions in millimetres.

FIG. 3 FAT EXTRACTION TUBE

Add 25 ml diethyl ether through the beaker used for weighing cream. Close the tube with the cork (or stopper) which is wetted with water before insertion, and shake vigorously for one minute. It is essential that the cork (or stopper) be wetted before each insertion and washed with solvent during each removal. Also, to avoid spurting of the solvent, a slightly reduced pressure should be induced in the tube by cooling before each removal. Rubber stoppers shall not be used.

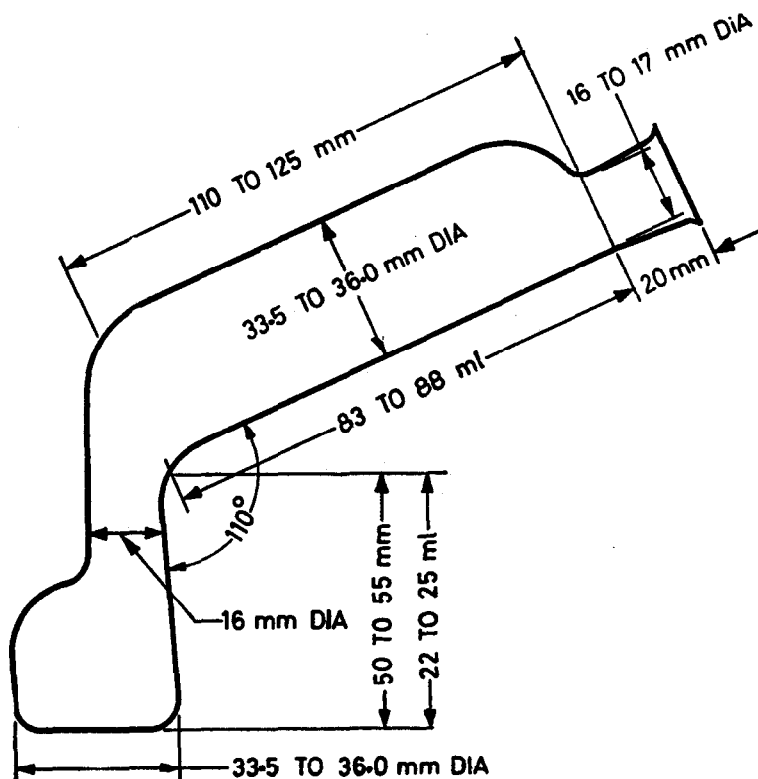


FIG. 4 MOJONNIER FAT EXTRACTION APPARATUS

Open the tube and add 25 ml of light petroleum; close the tube and shake vigorously for one minute. Allow the tube to stand on the flat bottom of the lower bulb until the ethereal layer is clear and completely separated from the aqueous layer, usually for not less than 30 minutes, or centrifuge until clear. Examine the tube to see if the junction of the liquid is at the lower end of the narrow neck of the tube. If it is below this, it should be raised by the addition of a little distilled water run down the side of the tube.

Carefully decant, as much as possible, of the supernatant layer into a suitable flask containing 2 glass beads. Decantation should be done by gradually bringing the cylindrical bulb of the tube into a horizontal position. When the maximum possible supernatant liquid has been poured off, wash the outside of the neck of the tube and the cork or stopper with mixed solvent, collecting the rinsings in the flask. With the Mojonnier

tube in a vertical position, wash the inside of the neck with 4 to 5 ml of mixed solvent, and decant.

Repeat the extraction of milk residue and the subsequent operations but using 15 ml of diethyl ether and 15 ml of petroleum.

Finally, repeat the extraction and subsequent operations once more with 15 ml diethyl ether and 15 ml petroleum.

4.2.3.3 Distil carefully the solvent from the flask collected under **4.2.3.1** or **4.2.3.2** and completely remove the solvent. Wipe the flask and dry the residual fat in the oven at 98° to 100°C for one hour, taking precaution to remove all traces of volatile solvent and cooling the flask to room temperature in a desiccator with efficient desiccant.

Extract the fat from the flask with successive 15 ml light petroleum. After the first addition, the flask should be warmed and the solvent swirled round the sides until all the fat appears to be in solution. Allow any sediment to settle and carefully decant the solution without disturbing the sediment through lightly packed cotton wool in a small funnel, to a weighed flask containing two glass beads. Repeat at least twice. Finally, rinse the neck of the flask with light petroleum, and after allowing the flask to stand, decant. As described above, distil off the solvents, heat the flask in the oven, allow to cool and weigh. Repeat heating in oven, cooling and weighing until successive weighings do not show a loss in weight by more than one milligram.

Simultaneously when the above procedure is carried out, make a blank determination with 1 to 2 ml water in place of cream. Use a similar extraction apparatus, the same reagents and the same technique throughout. The difference in weight before and after the petroleum extractions, after correcting for the blank, is the weight of the fat contained in the weight of cream taken.

4.2.3.4 Calculation — Calculate the percent of fat by weight in the cream to three significant figures. The maximum deviation between duplicate determinations shall not exceed 0.2 percent of fat.

5. DETERMINATION OF TITRATABLE ACIDITY

5.1 Apparatus

5.1.1 Burette — with soda-lime guard tube.

5.1.2 Porcelain Dishes — white, hemispherical, of approximately 60-ml capacity.

5.1.3 Stirring Rods — of glass, flattened at one end.

5.2 Reagents

5.2.1 Standard Sodium Hydroxide Solution — 0.1 N. Prepare a concentrated stock solution of sodium hydroxide by dissolving equal parts of sodium hydroxide (sticks or pellets) in equal parts of water in a flask. Tightly stopper the flask with a rubber bung and allow any insoluble sodium carbonate to settle down for 3 to 4 days.

Use the clear supernatant liquid for preparing the standard 0.1 N solution. About 8 ml of stock solution is required per litre of distilled water. The solution should be accurately standardized against acid potassium phthalate or oxalic acid.

5.2.2 Phenolphthalein Indicator Solution — Dissolve one gram of phenolphthalein in 110 ml rectified spirit (*see* IS: 323-1959*). Add 0.1 N sodium hydroxide solution until one drop gives a faint pink colouration. Dilute with distilled water to 200 ml.

5.2.3 Rosaniline Acetate Stock Solution — Dissolve 0.12 g of rosaniline acetate in approximately 50 ml of rectified spirit (*see* IS: 323-1959*) containing 0.5 ml of glacial acetic acid. Make up to 100 ml with rectified spirit.

5.2.3.1 Bench solution — Dilute 1 ml of the stock solution to 500 ml with a mixture of rectified spirit (*see* IS: 323-1959*) and distilled water in equal proportions by volume.

NOTE — The stock solution and the bench solution should be stored in dark-brown bottles securely stoppered with rubber bungs.

5.3 Procedure — Weigh 10.0 g cream into each of two white porcelain basins of approximately 60-ml capacity; add to both, 10 ml of water, and stir to disperse the cream. Prepare from one dilution a colour control by adding and stirring 2 ml dilute rosaniline acetate solution. Stir 2 ml phenolphthalein solution into the other dilution and, while stirring vigorously, add as rapidly as possible sodium hydroxide solution, from a 10-ml burette fitted with a soda-lime guard tube, until the colour matches the pink colour of the control. The titration shall be preferably done in north daylight or under illumination from a daylight lamp.

5.4 Calculation — Express the titrable acidity in terms of 'lactic acid', g/100 g of cream.

$$\text{Titrable acidity (as lactic acid), percent by weight} = \frac{9 AN}{W}$$

where

A = volume in ml of the standard sodium hydroxide required for titration,

*Specification for rectified spirit (*revised*).

N = normality of the standard sodium hydroxide solution, and
 W = weight in g of cream taken for the test.

6. PHOSPHATASE TEST

6.1 Apparatus

6.1.1 *Lovibond All-Purpose Comparator with Stand*

6.1.2 *Standard Discs* — giving 0, 6, 10, 18, 42; or 0, 6, 10, 14, 18, 25, 42 readings.

6.1.3 *Fused Glass Cells* — 25 mm.

6.1.4 *Test-Tubes* — 15×1.9 cm with ring at 10 ml and fitted with rubber stoppers.

6.2 Reagents

6.2.1 *Buffer Solution* — 3.5 g of sodium carbonate analytical reagent grade (see IS : 296-1951*), and 1.5 g of sodium bicarbonate analytical reagent grade (see IS : 491-1954†) dissolved in one litre of water.

6.2.2 *Substrate-Disodium p-Nitrophenylphosphate* — not less than 95 percent pure.

6.2.3 *Buffer Substrate* — Transfer 0.15 g substrate into a 100-ml measuring cylinder or stoppered graduated flask and make up to the mark with the buffer solution. The solution should not be stored for long periods but may normally be kept in a refrigerator for up to one week. The solution is practically colourless; when viewed through a 25-mm cell in the all-purpose comparator, it should give a reading of less than 10 units on the disc.

6.3 *Procedure* — Fill 10 ml (or 5 ml) of the buffer substrate solution into test-tubes (see 6.1.4) and bring to 37° to 38°C on a water-bath. Add 2 ml of the cream to be tested (one millilitre if 5 ml buffer substrate is used); close the tubes with rubber stoppers and invert to mix. Prepare in the same way a blank from cream of the same type; it should be heated for one minute at least at 85°C . Incubate all the tubes at 37° to 38°C . Read the yellow colour after 30 minutes, put them again into the bath, and take a second reading after incubation for a further 90 minutes. The yellow colour is read in a Lovibond all-purpose comparator on a resazurin stand, fitted with the disc calibrated in microgram *p*-nitrophenol. The blank is placed on the left of the stand and the sample on the right. Readings are taken by looking down on to the two apertures with the

*Specification for anhydrous sodium carbonate, pure and analytical reagent.

†Specification for sodium bicarbonate, refined (*tentative*).

comparator facing a good source of north daylight; the disc is revolved until the sample is matched; readings falling between two standards are recorded to the nearest reading.

6.4 Interpretation of Results

<i>Disc Reading After 30-Minute Incubation</i>	<i>Interpretation of Pasteurization</i>
0 or trace	Proper
6	Doubtful
10 or over	Under
<i>Disc Reading After 2-Hour Incubation</i>	
0 to 10	Proper
Over 10	Under

The 30-minute test will reveal any serious fault in pasteurization, but to enable minor errors to be detected, readings shall be taken after further incubation for 90 minutes.

7. DETERMINATION OF STANDARD PLATE COUNT

7.0 The standard plate count gives an estimate of the number of viable bacteria present in cream. Although the method is expensive and time-consuming, it has been widely used for assessing the hygienic conditions of production and handling of cream and for grading purposes. The principle and application of the method are the same as in the case of milk.

7.1 Apparatus

7.1.1 Bacteriological Pipettes, Sterile — of 1 ml and 11 ml nominal capacities.

7.1.2 Dilution Bottle — made of heat resistant glass (preferably borosilicate glass) of the following capacities:

- a) 150 ml, with mark at 99-ml level; and
- b) 25 ml, with mark at 9-ml level.

7.1.3 Petri Dishes — with outside dish diameter 100 mm, inside dish diameter 91 mm, and depth 15 mm. The exterior and interior surfaces of the bottom portion should be flat and free from bubbles, scratches or other defects.

7.1.4 Hot Air Oven — capable of giving uniform and adequate temperatures, equipped with a thermometer, calibrated to read up to 220°C, and with vents suitably located to assure prompt and uniform heating.

7.1.5 Autoclave — capable of providing uniform temperatures within the chamber up to the sterilizing temperature of 121°C, equipped with accurate mercury-filled thermometer with bulb properly located so as to register the minimum temperature within the sterilizing chamber (with or without temperature recording instrument), pressure gauge and properly adjusted safety valve.

7.1.6 Incubator — either water-jacketed or anhydric type, electrically-heated, thermostatically controlled, and provided with shelves so spaced as to assure uniformity of temperature. The incubator shall be maintained at 37° + 0.5°C.

7.2 Reagents

7.2.1 Dilution Water — Dissolve 34 g potassium dihydrogen phosphate (KH_2PO_4) in 500 ml distilled water; adjust to pH 7.2 with 1 N sodium hydroxide solution and make up to one litre with distilled water. Dilute 1.25 ml of this stock phosphate buffer solution with water to one litre.

7.2.2 Media — Use a plating medium of the composition described below. Preferably prepare plating medium from dehydrated base stock.

Tryptone	5.0 g
Yeast extract	2.5 g
Glucose (dextrose)	1.0 g
Agar, bacteriological grade (see Note)	15.0 g
Water, distilled	1 000.0 ml
Final pH	7.0 ± 0.1

NOTE — Granulated or chopped shreds practically free from thermophilic bacteria shall be used.

7.3 Procedure

7.3.1 Mixing the Samples — Mix samples of cream thoroughly by shaking the container through 25 complete up-and-down movements of about 40 cm in 7 seconds. In the case of filled containers invert them repeatedly until the contents are homogeneous.

7.3.2 Preparing Dilutions and Plating — Weigh 1 g or 11 g cream in an accurate balance and transfer aseptically into sterile 9 ml or 99 ml dilution blanks to give 1:10 or 1:100 dilution. After thoroughly mixing the diluted sample, prepare further dilutions using one millilitre pipettes. Where solid content of samples exceeds 20 percent, preferably prepare initial

1:10 dilutions by weighing test portions into dilution blanks. Use of dilution blanks heated to 35°-40°C facilitates cream dispersal therein.

When measuring diluted cream, hold the pipette at an angle of about 45° with tip touching inside bottom of petri dish or inside neck of dilution bottle or flask. Allow 2 to 3 seconds for the liquid to drain from the one millilitre graduation mark to apparent rest point in tip of pipette, and then touch pipette once against a dry spot on the glass. Do not blow out the drop in the tip of the pipette.

Transfer one millilitre of each of the required dilutions into duplicate petri dishes.

7.3.3 Pouring Plates and Incubation — Introduce 10 to 12 ml melted and cooled medium at 44°-46°C into each petri dish. Mix the medium with test portion by tilting and rotating the petri dish gently. Allow the medium to cool down and solidify in 5 to 10 minutes on a level surface. Invert the plates and place them in the incubator maintained at $37^{\circ} \pm 0.5^{\circ}\text{C}$ for 48 hours.

7.3.4 Counting of Plates — After the incubation period, count all the plates showing colonies between 30 and 300 and free from spreaders; a suitable colony counter or other device may be used. Note the colony counts and the dilution in each plate.

7.4 Recording Counts — Multiply the number of colonies or the average of counts in duplicate plates by the reciprocal of the dilution used. Express the results as standard plate count per millilitre or per gram of cream.

7.5 Interpretation — The following standards are suggested as a guide for grading of cream:

a) *Pasteurized Cream* (at the plant in the final container) — The standard plate count per ml (or g) should not exceed 60 000.

b) *Raw Cream*

SPC/ml (or g)	Grade
Below 400 000	Very good
Between 400 000 and 2 000 000	Good
Between 2 000 000 and 10 000 000	Fair
Over 10 000 000	Poor

NOTE — The general precautions and hints given in respect of plating, incubation, counting of plates and other details of technique for standard plate count of milk are applicable in the case of cream also [see IS : 1479 (Part III)-1962*].

*Methods of test for dairy industry : Part III Bacteriological analysis of milk.

8. DETERMINATION OF PRESUMPTIVE COLIFORM COUNT

8.1 Presence of excessive numbers of coliform bacteria in raw cream indicates insanitary conditions of production and handling, while in the case of pasteurized cream it serves as an index of post-contamination. The principle and limitations of the test and the procedure for estimation of coliform content described for milk [see 8 of IS : 1479 (Part III)-1962*] are applicable in the case of cream also. The test may be performed using either liquid or solid media. A suitable method using solid medium (violet red bile or desoxycholate agar) is described below.

8.2 Apparatus

8.2.1 Sample Bottles — sterile size, 50 ml or larger.

8.2.2 Pipettes — sterile, delivery 1 ml and 11 ml.

8.2.3 Petri Dishes — sterile, 10 cm outside diameter and about 1.5 cm depth.

8.2.4 Incubator — maintained at $37^{\circ} \pm 0.5^{\circ}\text{C}$.

8.2.5 Hot Air Oven

8.2.6 Autoclave

8.3 Reagents

8.3.1 Dilution Blanks, Sterile — 9 ml and 99 ml.

8.3.2 Plating Medium

8.3.2.1 Violet red bile agar — Prepare the medium preferably from dehydrated base (or from ingredients) consisting of 0.3 percent yeast extract; 0.7 percent peptone; 0.15 percent bile salts; one percent lactose; 0.5 percent sodium chloride; 1.5 percent agar; 0.003 percent neutral red; and 0.000 2 percent crystal violet meant for use in bacteriological work in water, with final pH 7.4 ± 0.1 . After complete rehydration, cool to 42° to 44°C before pouring plates. After solidification of the medium in plate, add cover layer of the medium. Preferably prepare the medium shortly before use; otherwise sterilize by autoclaving at 121°C for 15 minutes before use.

8.3.2.2 Desoxycholate (lactose) agar — Prepare the medium preferably from dehydrated base (or from ingredients) consisting of (a) one percent polypeptone, one percent lactose, 0.5 percent sodium chloride, 0.2 percent sodium citrate, 0.05 percent sodium desoxycholate, 1.5 percent agar, and 0.003 percent neutral red meant for use in bacteriological work in water with final pH 7.1 ± 0.1 ; or (b) one percent peptone meant

*Methods of test for dairy industry : Part III Bacteriological analysis of milk.

for use in bacteriological work, one percent lactose, 0.1 percent sodium desoxycholate, 0.5 percent sodium chloride, 0.2 percent dipotassium phosphate, 0.2 percent ferric ammonium citrate, 1.5 percent agar, and 0.003 percent neutral red (meant for use in bacteriological work in water with final pH 7.3 ± 0.1). After complete rehydration, cool to 42° to 44°C before pouring plates. After solidification of the medium in plate, add cover layer of the medium. Preferably prepare the medium shortly before use; otherwise sterilize by autoclaving at 121°C for 15 minutes.

8.4 Procedure

8.4.1 Dilution — Mix the sample of cream thoroughly by shaking the container and prepare 1:10 dilution by transferring 1 g (or 1 ml) into 9 ml dilution blank or 11 g (or 11 ml) into 99 ml dilution blank (see 7.3.1 and 7.3.2). Prepare 1:100 or higher dilutions as required.

8.4.2 Pouring Plates — With a sterile pipette, transfer 1 ml of each dilution into duplicate petri dishes. In the case of sample where the coliform content is expected to be low, transfer 5 ml or 1:10 dilution, or 1 g of the cream, directly into the petri dishes. Pour 10 to 15 ml violet-red bile or desoxycholate agar, previously melted and cooled at 42° to 44°C . In case 5 ml or 10 ml of the sample or the dilution has been inoculated into the plates, 20 to 25 ml of the medium should be added. Mix the contents thoroughly by gentle tilting and rotation of the plates. After mixture has solidified, pour another layer of 5 to 6 ml of the same medium and spread it evenly to cover the surface completely.

8.4.3 Incubation — Allow the medium to set. Invert and incubate petri dishes at $37^{\circ} \pm 0.5^{\circ}\text{C}$ for 24 hours.

8.4.4 Counts — At the end of the incubation period, remove the plates from the incubator and examine them for the presence of typical colonies of coliform bacteria (dark red colonies measuring at least 0.5 mm in diameter). The presence of 3 or more typical colonies of coliform bacteria in a plate should be interpreted as a positive coliform test in the particular dilution. Count all such colonies and report the results as coliform counts per ml (or g) of cream.

8.4.5 Interpretation — The following tentative standards are suggested for judging the quality of cream:

	Coliform Count per ml (or g) of Cream	Grade
Raw Cream	Not more than 100	Satisfactory
Pasteurized Cream	Not more than 10	Satisfactory

(Continued from page 2)

<i>Members</i>	<i>Representing</i>
GOVERNMENT ANALYST	Government of Madras
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MILK COMMISSIONER	Milk Commissioner, Madras
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DR S. S. PHATAK	Technical Standardization Committee, Foodstuffs (Ministry of Food & Agriculture)
SHRI RAM SARUP	Indian Council of Agricultural Research, New Delhi
DR ARUN KUMAR SEN GUPTA	Milk Commissioner, West Bengal
DR R. S. SRIVASTAVA	Public Analyst, Government of Uttar Pradesh
SHRI JAMES N. WARNER	In personal capacity (Allahabad Agricultural Institute, Allahabad)

INTERNATIONAL SYSTEM OF UNITS (SI UNITS)

Base Units

Quantity	Unit	Symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Thermodynamic temperature	kelvin	K
Luminous intensity	candela	cd
Amount of substance	mole	mol

Supplementary Units

Quantity	Unit	Symbol
Plane angle	radian	rad
Solid angle	steradian	sr

Derived Units

Quantity	Unit	Symbol	Definition
Force	newton	N	1 N = 1 kg.m/s ²
Energy	joule	J	1 J = 1 N.m
Power	watt	W	1 W = 1 J/s
Flux	weber	Wb	1 Wb = 1 V.s
Flux density	tesla	T	1 T = 1 Wb/m ²
Frequency	hertz	Hz	1 Hz = 1 c/s (s ⁻¹)
Electric conductance	siemens	S	1 S = 1 A/V
Electromotive force	volt	V	1 V = 1 W/A
Pressure, stress	Pascal	Pa	1 Pa = 1 N/m ²

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